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SYBIL ELLEN NATHANS DUCHIN

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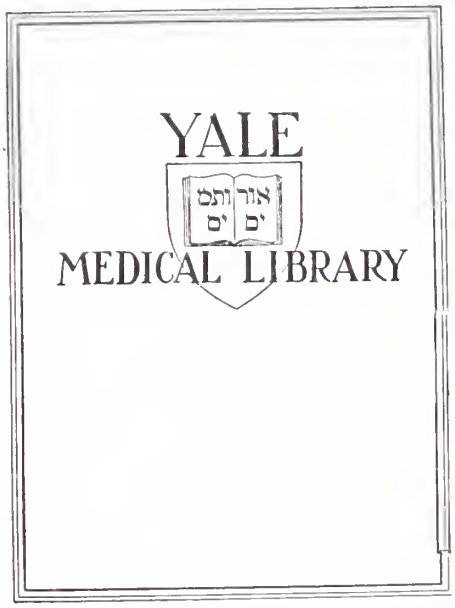
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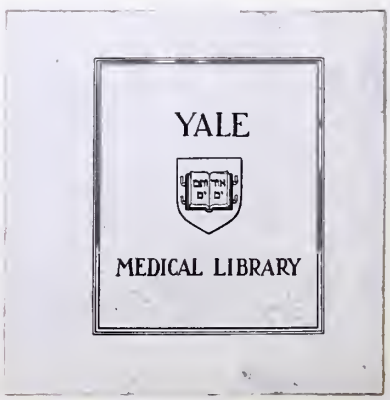
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EFFECT OF FUROSEMIDE AND HYPOKALEMIA ON THALLIUM-201 UPTAKE IN CANINE LEFT VENTRICULAR MYOCARDIUM

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A THESIS SUBMITTED TO
THE YALE UNIVERSITY SCHOOL OF
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FOR THE DEGREE OF
DOCTOR OF MEDICINE

1977

DEDICATED

WITH ALL MY LOVE

TO MY MOTHER, FATHER, SISTER

AND ESPECIALLY TO MY HUSBAND

ARTHUR

WHOSE FAITH NEVER WANED

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I. INTRODUCTION

In the last decade the need to image the heart has led to the investigation of various ions that could serve as cardiac imaging agents in healthy and in non-functioning cardiac tissue and provide prognostic information allowing earlier medical or surgical intervention in patients with acute myocardial infarction.

These imaging agents have been found to function by mimicking the action of the normal in vivo cations (ie. K^+). Thallium (to be discussed in greater detail later) has been shown to function as an excellent K^+ analogue in many systems (1) and to serve as an excellent myocardial imaging agent for the relative quantitation of infarct size (2,3). Gustin (4) in 1975 felt that Cesium-129 was also an accurate myocardial imager for the localization and relative quantification of acute myocardial infarction. A future goal would be to accurately quantify infarct size using these techniques. To be able to use cardiac imaging in a quantitative manner, we must understand factors affecting the uptake of the imaging agent thallium.

It is well established that diuretic drugs effect cationic balance in the renal tubule (5,6). Only a few studies have shown an extrarenal site of diuretic drug action. Sellers in 1975 (7) provided some of the earliest evidence for diuretics exerting a direct effect on the myocardium.

He found that furosemide and ethacrynic acid individually increased the digitalis induced myocardial loss of potassium from the dog heart as measured by arterial-venous differences. In addition there is evidence that ethacrynic acid and triamterene act on the erythrocyte Na^+,K^+-ATPase as well as the renal tubule cells. With these leads on the extrarenal sites of action of diuretics as well as with the many known changes in cationic balance caused by diuretics, we felt it was reasonable to study one of these diuretics in relation to the myocardium in more detail.

It is the goal of this investigation to study the potent kaliuretic diuretic, furosemide, in an in vivo mammalian system, to see if its acute or chronic administration under an eukalemic or chronic hypokalemic environment, affects the myocardial tissue concentration and kinetics of thallium-201 under these various conditions. We anticipate that the information derived from this study may help to contribute to the understanding of the mechanisms of the interrelationship between potassium dynamics, cationic enzyme systems, and furosemide; in addition, it may eventually contribute to the knowledge needed for accurately sizing myocardial infarcts with ^{201}Tl.

II. IMAGING AGENTS - THALLIUM-201

Thallium, a metallic element in group IIIA of the periodic table (2), has properties that closely resemble potassium in biologic systems, suggesting to Kawana that tracer quantities of 201 Tl may be used to image the myocardium(2).

Available evidence suggests that the movement of thallous thallium and potassium in animals is related (1). Mullins and Moore (8) found that both the influx and efflux patterns of thallium were very similar to potassium when frog muscle was used as a test tissue. Gehring and Hammond in 1964 (9) presented evidence indicating that the mechanism associated with the active transport of potassium into rabbit erythrocytes also transported thallium. All the experiments suggested a great similarity in the ionic movements of thallium and potassium. By determining and comparing the disappearance of ^{204}Tl and ^{42}K from plasma and the uptake of these ions by tissues as a function of time, the degree to which these ions are interchangeable was characterized(1). The concentrations of ^{204}Tl and ^{42}K in heart, lung, muscle, intestine, skin, brain, liver and spleen as a function of time were similar. The high initial concentration of these ions in the heart and lung immediately following injection were related to blood flow (1). ^{201}Tl concentrates in the myocardium in relation to the distribution of regional perfusion under normal flow, and partial and total occlusion

of the coronary artery(2). Thallium appears to concentrate in the myocardium to a greater degree than potassium and rubidium, 2.08 % dose compared to 1.25 % dose and 1.15 % dose respectively at 10 minutes, which is the time most commonly employed for imaging(2).

Maximal myocardial and renal concentration occurs at 10 minutes. The maximal total organ content of thallium in the myocardium is 3.7 % of the injected dose at 10 to 25 minutes. The half life of ^{201}Tl in the myocardium is in two parts: 4.4 hours (78%) and 40 hours (22%). The half life of ^{201}Tl from the blood is less than one minute(10).

Gehring and Hammond(1) felt that since the concentration of ^{204}Tl in the heart and lung were consistently greater than that of ^{42}K, these tissues probably had a slightly greater affinity for the ^{204}Tl, and although the uptake of ^{204}Tl and ^{42}K by tissues may be related, there are sites within the cell having a greater affinity for thallium than for potassium(1). The kinetic analysis of simultaneous plasma decay data for ^{204}Tl and ^{42}K suggested that the uptake of these ions by tissues are similar. An analysis of the organs of the body suggested that handling of ^{204}Tl and ^{42}K by the kidney, testes and intestine was different(1).

Since the uptake of potassium is in part dependent on an active trasport system and since the uptake of thallium and potassium by tissue compartments is related, thallium probably is also actively transported into cells. It has

been shown in rabbit erythrocytes that the movement of thallium into the cells results from the active transport of thallium by a mechanism responsible for the active transport of potassium(9). Evidence strongly suggested that Na^+,K^+ activated ATPase isan essential component of the active K transport mechanism(11). The finding that thallium can substitute for potassium in causing activation of this enzyme provided further evidence that the uptake of thallium by all cells in part occurs via the active transport system associated with the uptake of potassium(1). In addition, Britten and Blank in 1968 (12) found that thallium replaces potassium in activating the Na^+,K^+-sensitive ATPase of rabbit kidney with an affinity approximately ten times potassium for the potassium activating site. An additional property that thallium and potassium have in common is their limiting ionic conductances in water at 25°C (Tl^+, 74.7 and K^+, 73.54), indicating that the hydrated Tl^+ is almost identical in size to the hydrated K^+. It is undecided whether the substitution is by hydrated ions at the solvated site or by free ions interacting with an enzyme site. It has also been shown that thallium is handled like potassium by rat sartorius muscle and also that thallium behaves like potassium during electrical excitation of that tissue. These studies established that thallium is a substitute for potassium in vivo(12).

Potassium-43 is not considered ideal for imaging because of its high photon energies (373 and 619 kev), short shelf

life (t 1/2 = 22.4 hours), and high cost. Thallium-201, with all the evidence for its functioning as an excellent analog of potassium in vivo, on the other hand, is considered ideal to use as an imager since it emits relatively low energy gamma photons of 69-83 kev in 90% abundance plus gamma rays of 135 (2%) and 167 (8%) kev in 10% total abundance, giving lower total body radiation to the patient, has a half life of 73.5 hours, has a short time interval both between the injection and the optimal scintiscan (10-20 minutes) and between the onset of infarction and scintigraphic results (few hours)(2,3,13). Its low energy photon and X-ray emissions make the use of high resolution low energy collimators with either the rectilinear scanner or scintillation camera feasible to image the tracer while imaging K or Rb requires high energy low resolution collimators(14,15,16). Since the low energy X-ray emission from thallium-201 are more abundant and the resolution with the two high resolution scintillation camera systems is satisfactory with the narrower window, the X-ray is the optimal method of imaging thallium(2). Imaging with thallium-201 after an acute myocardial infarct gives a dose of 3.8 rads per mCi to the kidneys of man. 12% of administered activity is excreted with the urine and 6% with the feces(3).

During the acute phase of a myocardial infarction, both necrotic and ischemic tissue is demonstrated by thallium-201 since this ion concentrates in the myocardium in relation to the distribution of regional myocardial flow. Subsequent

formation of collaterals in previously ischemic regions leads to recurrence of thallium-201 uptake. Defect size depends on the time of the scintigraphic study after the onset of the infarct and must be considered if quantification of infarct size by thallium-201 is to be attempted. Disadvantages of thallium-201 scintigraphy are that no exact differentiation can be made between acute infarction and ischemia and that thallium-201 cannot distinquish between old and new infarction. Thallium-201 has the advantage of its use in early detection within 6 hours, and probably within minutes, after the onset of symptoms(17).

Thallium differs from potassium in that thallium is poisonous to mammals in a dose of 20 mg per kg (2).

III. FUROSEMIDE

Furosemide is a weak anthranilic acid derivative producing dilatation of renal vasculature and an increased blood flow rate especially in the renal cortex and a decreased rate in the outer medulla(18,19,20). After oral intake the diuretic response occurs within an hour. Given intravenously it occurs within 2-10 minutes with peak electrolyte response in 30 minutes. After intravenous administration, some furosemide is filtered at the glomerulus although more important is secretion by the proximal tubule, both of which give a combined excretion of two-thirds of the diuretic. The proximal tubule secretion is blocked by competitive inhibitors of proximal tubule organic acid transport. The remaining third is excreted in feces. Furosemide is bound to dog plasma protein 85-90%(19). The highest concentration of unchanged drug following ^{14}C furosemide is found in kidney, liver, plasma and lung. Heart, muscle and fat show less radioactivity per gram of tissue than seen per ml of plasma. Furosemide appears in urine intact (96-99% of total urinary radioactivity) within the first 1/2 hour after intravenous administration. In a 24 hour collection period, 80% of urinary radioactivity excreted by dogs remained unchanged(19). (Mechanisms of action to be discussed in section IV).

IV. MECHANISMS OF POTENT DIURETICS WITH EMPHASIS ON FUROSEMIDE AND POTASSIUM EXCHANGE

Many studies have shown that the active movements of Na^+ and K^+ are closely linked under most conditions, since inward K^+ transport occurs only with an outward movement of Na^+ ions. A membrane bound energy dependent ATPase (with a molecular weight between 300,000-400,000) is known to mediate the active movement of these cations through the plasma membrane of the renal tubular cell, red blood cell and myocardium and this enzyme is stimulated synergistically by internal Na^+ ions and external K^+ ions(11,20,21). Skou in 1960 (22) first demonstrated that this Na^+,K^+-ATPase is inhibited by ouabain.

It has been found that 3 Na ions bind on the inside and two K ions bind on the outside of the red blood cell membrane Na^+,K^+-ATPase ("pump") for each molecule of ATP hydrolyzed. This ATPase requires ATP hydrolysis at a rate dependent on external K^+ and internal Na^+ concentrations, and is specifically sensitive to inhibition by cardiac glycosides unlike other ATPases which do not depend on Na^+ and K^+ for their function and are probably associated with other cellular functions. Both Na^+ ions inside and K^+ ions outside are needed to stimulate the pump-enzyme because these Na^+ and K^+ ions' active transport in the red blood cell is coupled(21).

Levels of activity of Na^+,K^+-ATPase in a given tissue correlates well with the rates of active cation transport and pumping rate in the same tissue. The specific activity

of this enzyme from red blood cells is only 1/2000th of it in kidney preparation(21). The small distal tubular cell mass possesses an extremely high activity of Na^+,K^+-ATPase compared with other tubular structures as found by quantitative histochemical studies(20). The highest Na^+,K^+-ATPase activities were measured in the outer medulla largely made up of the thick ascending limbs of Henle's loop(23).

Glycosides given intraarterially produce natriuresis and reduction in renal concentrating ability with an inhibition of Na^+,K^+-ATPase activity in the cortex and medulla of infused kidney(23). The rate of binding of ouabain rather than its capacity for binding to outer red blood cell membranes Na^+,K^+-ATPase (and to other systems where ouabain is effective in inhibiting this ATPase, ie. myocardium), is depressed proportionally by increasing the external K^+ concentration(21,24,25). However, this increase in K^+ concentration eventually alters neither the peak inotropic effects of digoxin nor the myocardial concentration of ^3H-digoxin; it only slows the rates to its achievement(25). In addition, K^+ appears to decrease the rate of combination of ethacrynic acid with this transport system(26). There is much evidence that when the cell gets enough digoxin to inhibit the pump completely, the permeation of Na^+ and K^+ through the membrane is not entirely accounted for by passive "leakages". Ethacrynic acid partially inhibits this remaining Na^+ outflux and K^+ influx in digoxin treated cells through a digoxin-insensitive pump (ATPase) called Pump II. This pump is not affected

by the external K concentration(27). The digoxin-sensitive pump (ATPase) which exchanges Na^+ for K^+ is called Pump I(28).

The kinetic behavior of the active K influx in the presence of digoxin and ethacrynic acid is consistent with a model in which binding of K at one of the K-sensitive sites in the transport system reduces the affinity of the system for the ethacrynic acid and binding of a second ion further reduces the affinity(26). Another possibility is that digoxin does not compete for the K^+ or Na^+ sites on the ATPase but binds to an allosteric site on the cell membrane which leads to a conformational change on the ATPase enzyme which then inhibits its transport functions(29,30).

Furosemide, as well as other potent diuretics, has been found to affect cationic movement in systems other than the renal tubule, such as the red blood cell, salivary duct epithelium and the myocardium(7,31,32).

There are a number of different explanations existing, many of which are interrelated, about how furosemide causes natriuresis. Furosemide exerts its effect from the luminal surface of the tubule(33). Inhibition of ionic absorption from the ascending limb of the loop of Henle is one of the major effects of furosemide in the human kidney and this has the effect of reducing the diluting and concentrating ability of patients(18). Furosemide also leads to a metabolic alkalosis since more chloride is lost than bicarbonate, in addition to the increased loss of hydrogen and potassium(27).

Furosemide affects several transport processes in the

kidney. It is known to cause increased urinary loss of sodium, chloride, calcium, and magnesium by the same per cent as sodium and to a lesser extent potassium in man; these effects are independent of glomerular filtration rate(18).

On micro puncture and microperfusion studies on single nephron segment, Burg in 1973 (33) showed that in rabbit thick ascending limb of Henle's loop that the active transport of chloride is the driving force for ionic movement and that sodium ions passively followed the chloride. Furosemide, at a concentration of 10^{-5} or 10^{-6}M in luminal fluid, inhibits this chloride transport process only in the thick ascending limb in vitro and not elsewhere in the tubule, with a decrease in net NaCl absorption and potential difference at the lumen from -5 to 0. Concentrations of furosemide as high as 10^{-4}M whether placed in the bath and/or the lumen had no definite effect on the proximal convoluted tubules or cortical collecting tubules(33). Natochin in 1976 (44) working with frog skin found that furosemide decreases chloride permeability rather than inhibiting the chloride pump as found by Burg(33), then leading to the inability of the cells to reabsorb the sodium(34).

Since the thick ascending limb is the major site of NaCl reabsorption in the distal nephron, inhibition of its function could lead to a greater diuresis than a comparative inhibition limited to the proximal tubule(33).

Schmidt and Dubach in 1970 (20) reported that furosemide inhibited Na^+,K^+activated ATPase in the ascending limbs

of Henle's loop and distal tubules of rat in the same areas where the Na^+,K^+ATPase is found to be most abundant and most likely mediates Na transport, and that it was unclear if any relation between this ATPase and the active chloride transport existed(20,33).

Most investigators believe in a direct influence of furosemide on cell metabolic pathways linked to cation transport by directly inhibiting renal oxidative metabolism(7,20,35,36). In vivo and in vitro studies revealed a depressed oxygen uptake in renal slices after 10^{-4} M furosemide to about the same extent as 10^{-3} M ouabain, both being high doses. A competitive inhibition of energy production was noted(20). Manuel in 1976 (36) found that both ethacrynic acid and furosemide inhibited oxidative phosphorylation in the rat kidney in vitro by inhibiting electron transport through phosphorylation site II. Biochemical bypass of site II significantly alleviated the respiratory inhibition by both agents. Both diuretics caused a reduction of flavoproteins and an oxidation of the cytochromes.

Kessler in 1969 (37) demonstrated in dogs that furosemide caused a natriureses of 30 to 40% of the filtered load of sodium. Cortical concentration of nucleotides were unaffected whereas in the medulla, ATP and ADP rose in a similar way as found after ouabain application. Thus furosemide appears either to stimulate ADP and ATP production or, more likely, to inhibit medullary ATP utilization. According to Kessler, reduction of the Na^+,K^+ATPase may explain these results with

this ATPase also acting as the renal receptor for furosemide.

In 1956, Glynn (38) demonstrated that the passive movements of Na^+ and K^+ ions deviated from the values expected of independent leaks of each cation down its own electrochemical gradient. This discrepancy has been generally attributed to exchange diffusion (which is an equal flux of a single ion species in both directions across the membrane mediated by the same carrier that cannot bring about a net ion transport) and there is some evidence that at least part of the ouabain-insensitive Na^+ movements in red cells results from this process(39).

A second possible explanation is that ouabain-insensitive fluxes of Na^+ and K^+ are not independent but are coupled. Several findings suggest that Na^+ and K^+ may be cotransported into the red cell by the same mediated process. First, Na^+ influx is stimulated by the presence of K^+ ions in the medium and this increment in Na^+ influx is prevented by furosemide. Similarly, the K^+ influx, 20-25% of which persists in the presence of ouabain, is doubled or tripled when Na^+ ions are added to the medium and this increment in K^+ influx is abolished by furosemide. The synergism observed between the inward movement of Na^+ and K^+ in the presence of ouabain suggests that each ion facilitates the inward transport of the other. Moreover, the inhibition of the K^+-stimulated Na^+-influx as well as the Na^+-stimulated K^+-influx by furosemide indicates that these mutually stimulated cation fluxes occur through the same pathway, which is sensitive to furosemide(23,32,39,40).

Since the magnitude of the furosemide sensitive influxes of Na^+ and K^+ are not significantly different (0.39 and 0.32 ueq/ml cell/h respectively) the data are consistent with an inwardly directed cotransport mechanism, defined as a stoichiometric coupling between the inward movement of Na^+ and K^+ and for which each ion is the preferred but not the obligatory substrate. K^+ influx in the presence of ouabain does not increase in direct proportion to the external concentration of K^+ but tends to reach a limiting value as the concentration is increased(41). But in the presence of furosemide, the ouabain-insensitive K^+ influx shows a linear dependence of external K^+ concentration. Thus furosemide eliminates a component of K^+ influx that shows the saturation kinetics typical of a facilitated diffusion process. Omission of Na^+ from the medium likewise elimiates a saturable component of K^+ influx and the magnitude of the Na^+ sensitive component was nearly equal to the component inhibited by furosemide(41,42).

Wiley (32) feels that one implication of this linkage observed between inward movement of Na^+ and K^+ is that a minimum of two transport sites are present on the outward-facing aspect of the furosemide-sensitive pathway. While these two sites show specificity for Na^+ and K^+, respectively, it is likely that the specificities are not absolute and that each ion is not an obligatory cosubstrate, Thus the furosemide-sensitive mechanism may transport not only Na^+-K^+ pairs but also K^+-K^+ pairs, if Na^+ is unavailable for combination with its transport site. The action of furosemide is not

confined to inhibition of cation co-transport but furosemide also inhibits active cation fluxes by 10-15% as measured either by active K^+ influx or active Na^+ efflux (32).

Cotransport mechanisms are now recognized in a variety of tissues in which a coupled movement of Na^+ ions together with amino acids or sugar can lead to net movement of these solutes. Such movements can occur even against a concentration gradient and it seems likely that the energy for uphill solute movement is derived from the coupled movement of Na^+ down its gradient (43). An analogous cotransport of Na^+ plus K^+ ions should likewise result in inward K^+ movement against the concentration gradient of this ion. Thus, this study demonstrated that furosemide inhibited the ouabain-insensitive efflux of both Na^+ and K^+ by an action that is not simply due to an inhibition of exchange diffusion (32).

The clinical use of furosemide during an acute myocardial infarction with pulmonary congestion is well established in medical practice. In an acute myocardial infarction, the use of furosemide gives clinical relief of symptoms of pulmonary congestion frequently preceding any demonstrable diuretic effect, suggesting that extrarenal factors may also be involved (44). These acute cardiovascular effects are due to changes in the effective circulating volume, electrolyte alterations, direct effects on the vascular beds and secondary reflex effects (34,45). After an intravenous dose of furosemide, venous capacitance rises, limb vascular resistance declines at 5 minutes, along with a decrease in left ventricular

filling pressure from about 20 to 14 mm Hg, a decrease in right atrial pressure and cardiac output and a decrease in pulmonary arterial pressure due to increase in diameter or a relaxation of the pulmonary veins all before diuresis occurs. A redistribution in blood volume with much of it going to the limbs seems to be important at this early stage. Intact renal function is not necessary for this early effect. By 15 minutes a significant increase in glomerular filtration rate and PAH clearance which is consistent with a rise in renal plasma flow occurs, and a significant diuresis begins which further decreases the effective plasma volume. Between 30 and 60 minutes a further reduction in left ventricular filling pressure occurs this time due to the decrease in plasma volume due to the brisk diuresis (44,45).

Mierzwiak in 1975 (34) investigated the effect of large doses of furosemide (30 mg/kg) on the contractility of the heart of a vagotomized dog preparation where the blood pressure and heart rate were held constant. He found that after large doses of furosemide, no acute effects on the dog left ventricle (LVEDP in cm H_2O, maximum dp/dt in mm Hg/sec, stroke work) occurred, even after vagotomy and beta blockade. Therefore, any cardiovascular effects seen in man after furosemide administration are most likely related to changes in effective circulating blood volume, direct affects on vascular beds, electrolyte alterations and reflex effects rather than to any direct myocardial effects(34).

An interesting study by Horrobin in 1974 (46) showed that furosemide can inhibit both the potentiation and

depressive responses that arterial and arteriolar smooth muscles have in the presence of low (50 ng/ml) and high (200 ng/ml) concentrations of prolactin, respectively, when exposed to noradrenaline and to angiotensin. Furosemide returned the heart rate almost to starting values whether or not they had been increased (with low concentration prolactin) or decreased (with high concentration prolactin which is in the range of many patients, male and female, in renal failure or congestive heart failure) (56).

An important investigation closely linked to the present investigation was conducted by Seller in 1975 (7). Using therapeutic doses of digitalis, and potent diuretics in in vivo dog preparations, he found that the potassium sparing diuretics, triamterene and amiloride, reduced the digitalis-induced increase in myocardial loss of potassium (reduced the V-A difference), while the kaliuretic diuretics, furosemide and ethacrynic acid significantly increased the digitalis-induced myocardial loss of potassium (as measured by increased V-A difference). This finding contradicts the previous one by Mierzwiak (34) who felt that furosemide had no direct effect on the myocardium although Seller did not test furosemide alone nor did he monitor cardiac contractility along with the $(V-A)_K$.

The finding of a cardiac effect of diuretics supports the premise that there may be a common enzyme system responsible for K^+ transport across the renal tubular membrane, the red blood cell membrane and the myocardial membrane.

Triamterene and amiloride block renal Na^+-K^+ exchange due to a distal tubular site of action(47,48). Triamterene also stimulates both Na^+-K^+-dependent and Na^+-K^+-independent red blood cell membrame ATPase (49). In contrast, ethacrynic acid and possibly furosemide (potent diuretics due to their renal tubular sites of action (50)), have been shown to inhibit the digitalis-independent,Na^+,K^+-dependent membrane ATPase called "Pump II"(41,51), which has been shown to occur in red blood cell membrane, as previously discussed. It is possible that "PumpII" may also be present in myocardial membrane or be one of multiple mechanisms responsible for transporting potassium back into the myocardial cell, one which is digitalis-sensitive (Na^+,K^+-dependent ATPase) and one which is digitalis-insensitive. The latter may be similar to "Pump II". Thus digitalis and these kaliuretic diuretics may have additive blocking action regarding the re-entry of potassium into the myocardial cell too (7).

Experiments combining ouabain and ethacrynic acid as discussed earlier resulted in a complete inhibition of fractional Na reabsorption and only a 46% inhibition of Na^+,K^+-ATPase activity. The complete inhibition of Na reabsorption at only a partial inhibition of this ATPase is probably caused by additional metabolic effects of ethacrynic acid. It was thus suggested that the Na^+,K^+-ATPase is not the only diuretic receptor (51). Several natriuretic drugs have been reported to have no influence on the Na^+,K^+-ATPase such as thiazides, acetazolamide, spironolactone, and xanthine derivatives (23). Several investigators have attempted the unfolding of the more intimate cellular mechanisms of furosemide

and other potent diuretics (18,31,51,52). Another enzyme possibly involved in the control of Na transport and water permeability is the renal adenyl cyclase(51). Under recent investigation is the link between furosemide and ethacrynic acid to cyclic 3',5'-adenosine monophosphate (cAMP) dependent system. In the Kidney adenyl cyclases with different sensitivities to hormones exist. An ADH sensitive adenyl cyclase is located primarily in the renal medulla and a PTH one in the cortex. They both increase cAMP intracellularly in the medulla and cortex respectively. PTH and cAMP both inhibit phosphate reabsorption and increase calcium absorption(18,51). ADH stimulates Na transport across the tissue by increasing the rate of Na entry into isolated toad bladder epithelial cells(18). Catecholamines can affect the kidney adenyl cyclase system. Beta adrenergic drugs, such as isoproterenol, induce an antidiuretic effect and alpha adrenergic drugs cause diuresis (by inhibiting ADHaction) all via changes in cAMP(23).

1mM furosemide and separately 1mM ethacrynic acid inhibited adenyl cyclase in homogenates of kidney cortex and inner medulla, while amiloride did not, In the cortex the furosemide induced inhibition of adenyl cyclase can be reversed by 1 U PTH and 0.1 mM isoproterenol. Cortical PTH normalized the inhibition of adenyl cyclase by ethacrynic acid but the ethacrynic acid does not allow the PTH to raise the cAMP levels as high as without the ethacrynic acid or with furosemide. Ethacrynic acid is not influenced by isoproterenol. These data indicate that in the cortex,

furosemide and ethacrynic acid may act at different sites of the renal adenyl cyclase system. In the inner medulla the furosemide induced inhibition of adenyl cyclase was partially antagonized by ADH, but 25% inhibition remained. ADH did not reverse ethacrynic acid inhibition of adenyl cyclase at all. In addition, isoproterenol was totally ineffective in antagonizing the inhibition of adenyl cyclase caused by either furosemide and/or ethacrynic acid and was without influence on adenyl cyclase in the absence of these diuretics. The different action of isoproterenol on the furosemide induced inhibtion of adenyl cyclases in the cortex and medulla suggests that the adenyl cyclases of both kidney zones are different. We may be looking at a mixture of cyclases with different sensitivities since furosemide does not lead to a water diuresis which might be expected if it is thought of as opposite to ADH in the adenyl cyclase system(51).

Ferguson (18) believes that these results suggest that ionic reabsorption in the kidney is controlled by a cAMP mediated system, which is inhibited by furosemide, since furosemide inhibits ADH and cAMP induced Na transport but not transport of Na in their absence in the resting condition, and ethacrynic acid reduces Na transport in the presence of ADH and in its absence, although not Na transport stimulated by cAMP (52).

Biochemical studies show that ethacrynic acid reduces intracellular levels of cAMP, both in the resting state and in the presence of ADH, whereas furosemide has no effect. However, furosemide has chemical similarities to cAMP,

($6X10^{-4}$M furosemide) displaces cAMP from specific cAMP binding proteins (in both rabbit muscle and toad bladder), inhibits the activity of a cAMP dependent protein kinase which phosphorylates histones in the presence of ATP, has similar pK's, melting points, size and structure, almost identical molecular weights and at physiologic pH 7.4, they co-chromatograph. These studies suggest that although these diuretics are chemically different, their similar pharmacological effects may be explicable by a biochemical link, possibly involving their effect on cAMP mechanisms (18,52). It is possible that the diabetogenic effects sometimes seen with the clinical use of furosemide may be due to its interference in the cAMP systems although there is no solid evidence for this (52).

Rupp in 1974 (53) felt that the diuretic effect of furosemide could be predicted from serum concentrations. At an intravenous dose of 1 gram furosemide (in 40 minutes), concentrations close to 10^{-3} M were found which with 10% protein binding was equivalent to 10^{-4} M. This concentration gave extrarenal effects on Na transport in toad bladder, frog skin, red blood cells, smooth muscle cells and caused a reversible reduction in hearing. At an infusion rate of 4 mg per minute (1 gram in 240 minutes) gave a maximum serum concentration below 30 ug per ml (10^{-5} M with protein binding) and at these concentrations no extrarenal effects were observed including no reduction in hearing at this concentration (53).

It is currently thought that potassium depletion is a serious hazard especially in patients in chronic heart failure since it seems to increase the risk of digoxin

toxicity. This depletion is usually attributed to diuretics (54). There have been several conflicting studies as to whether chronic diuretic therapy actually leads to hypokalemia and/or to the depletion of body potassium. Since total extracellular fluid space contains only 3% of the total body content of potassium, plasma levels may not accurately reflect total body or intracellular potassium status (55). Therefore, several groups (54,56) have used the technique of measuring total body potassium (T.B.K.) by counting radioactivity from the naturally occuring radionuclide ^{40}K, the amount of which bears a constant ratio to the stable isotope ^{39}K which comprises the bulk of potassium. Using a total body monitor, the number of counts obtained is related to the weight of the potassium present with an accuracy by $\pm 2.5\%$.

Anderson (56) in 1971 found no significant change in T.B.K. in hypertensive patients treated for 8 weeks with each of hydrochlorthiazide 50 mg twice a day and lasix 40 mg twice a day with an intervening placebo period for 8 weeks.

However, Dargie (55) found that 4 months of 40 mg lasix per day without K supplements given to patients with essential hypertension led to a significant decrease in T.B.K. and in plasma K (3.9 to 3.6), although after 12 months of this therapy the T.B.K. was found to be normal again with the plasma K staying at the decreased value. In addition, there was a significant decrease in chloride at 4 months with no further decrease at 12 months.

Healy in 1970 (57) reported moderate reductions in total exchangeable potassium (T.E.K.) of 247 meq (which equals 9.8 grams K) in hypertensive patients after 15 weeks treatment with 40 mg lasix twice a day. This decrease in T.E.K. is comparable to the reduction in mean T.B.K. seen in Dargie's study at 4 months. But Dargie's group returned to baseline at one year. Dargie feels that this reduction and then return in T.B.K. may be due to a compensatory decrease in urinary K losses despite the continuation of other effects of furosemide, ie. the hypotensive and increase in uric acid effect (55), since in a non-edematous patient, K excretion can be as low as 15-30 mEq per day with furosemide (56). He, therefore, concludes that there is a poor correlation between the slightly low serum K levels and T.B.K.

Edmonds in 1975 (58) felt that lasix has a rapid short action, so that a single dose would not act over much of the 24 hours, allowing possible retention of K to compensate for the excess loss. He was therefore not surprised that Dargie's study (55) had only small decreases in serum K and T.B.K. He usually found that with thiazides, and especially in younger patients with mild to moderate hypertension without acid-base disturbances, that serum K and T.B.K. remained normal. However, he found that some patients did have persistent hypokalemia (less than 3.4 meq/l). These patients tended to be older and had more severe hypertension. He found that with a persistent hypokalemia,

a 10% decrease in T.B.K. agreed with the two patients in Dargie's study with plasma potassiums of 3.2 mEq/l.

A possible mechanism for the decreaed potassium in the sicker patients may be that cardiac failure leads to tissue anoxia which causes the cell to be unable to accumulate K which in turn leads to K depletion. In addition, in cardiac failure, secondary hyperaldosteronism leads to increased urinary excretion of potassium (55, 58).

The final parameter of importance, (especially in the context of this study), to be presented here, is hypokalemia and K depletion induced by diet.

Poole-Wilson in 1975 (59) and Cameron in 1975 (60) each found, in rabbits made chronically K deficient by a low potassium intake for 20 days, that there was a reduced amount of potassium in the plasma and skeletal muscles (quadriceps) but no change in the amount of K in the left ventricle, although Cameron felt that in patients in heart failure, their myocardial potassium may be reduced. In addition, Blushke in 1976 (61) found that in potassium-depleted guinea pigs (on a K deficient diet for 12 days), the potassium concentration in the serum and skeletal muscle were significantly decreased by 33% and 11% respectively with no decrease in K concentration in the heart muscle, and rats on a K-deficient diet for 6-8 weeks produced a marked reduction in the K content of the serum (from 4.9 to 1.9 meq/l) and skeletal muscle(from 460 to 304 meq/kg) whereas K content of the heart muscle was only slightly, but not significantly, diminished (from 378 to 355 meq/kg).

Erdmann in 1971 (62) showed in guinea pigs that K-deficiency caused an increase in activity of the Na^+,K^+-activated ATPase of the heart muscle. Digitalis glycosides are supposed to inhibit the Na^+,K^+-activated ATPase and reduce the intracellular K concentration or delay the restitution of the intracellular ionic concentration. Bluschke (61) therefore, tested whether an inhibition of the ATPase by long term treatment with digitoxin might change the activity of the enzyme. He found in guinea pigs made hypokalemia with 12 days on a K deficient diet and others treated with 14 days of subcutaneous digitoxin (0.3 mg per kg), that the activity of the Na^+,K^+-ATPase of the heart was significantly enhanced by 40% (compared to Erdmann's 130% increase) and 30% respectively whereas this ATPase of the kidney and brain showed no significant change in activity compared to controls. During this period no relationship was found between duration of treatment and the size of the increase in activity. As the activity of the Na^+,K^+-ATPase is dependent on the K concentration and as cardiac glycosides are the specific inhibitors of this enzyme, the observed increase in activity may be explained by an adaptive response to an inhibition of the ATPase. The enzyme activity is expressed as a turnover of substrate per mg total protein. Therefore, an increase in activity may be caused by an enhanced turnover (which would mean a change of the specific properties of the enzyme) or by an increase in the amount of enzyme within total protein. After kinetic studies were performed, it was suggested that

the increase in activity may possibly be caused by an increase in the amount of enzyme as a result of an adaptive enzyme induction(61).

V. MATERIALS AND METHODS

A. RADIONUCLIDE

The radiopharmaceutical used was cyclotron produced ionic thallium-201 supplied as a sterile, pyrogen-free radiochemical in 0.9% saline at pH 5-6 in a specific activity of 1.0 mCi per ml. Its peak energies in tissue and blood samples were read in a well-type scintillation counter window of 60 to 100 kev. The half life of thallium-201 is 73.5 hours. All samples were counted within 6 hours of injection into the animal. All counts were corrected to the original time of counting of the thallium doses for the day of the experiment.

All calculations were normalized to a 20 kg ideal weight.

Calculation 1. For blood samples:

$$\frac{\text{CPM} \times \text{weight of animal in kg/20 kg}}{(\text{cc}) \times (\text{mCi injected corrected back to injection time})} = \text{CPM / cc-mCi}$$

Calculation 2. For tissue concentration of thallium-201:

$$\frac{(\text{CPM / g}) \times (100) \times (\text{weight of animal in kg / 20 kg})}{(\text{mCi-corrected for time}) \times (\text{standard CPM / mCi})}$$

$$= \% \text{ injected dose / g}$$

B. ANIMAL PROTOCOL

In all studies 15-25 kg mongrel dogs of both sexes were anesthetized with intravenous pentobarbital (25 mg/kg), intubated with a cuffed endotracheal tube, and ventilated on room air with a Harvard respirator adjusted to the dogs' weight. Each dog underwent a right thoracotomy in the fourth or fifth interspace. The pericardium was incised and retracted. A polyethylene catheter was placed in the left carotid artery to monitor blood pressure, and in the right carotid artery for arterial blood collection, and in the left external jugular vein for injection of the furosemide, thallium and saline. A catheter was placed into the right external jugular vein and passed into the coronary sinus under visual and tactile observation for collection of venous blood samples. A foley catheter was inserted for collection of urine samples for measurement of urine volume, electrolytes, and for the timing of diureses.

Simultaneous samples from the urine, carotid artery and coronary sinus were obtained twice during a control period. Carotid artery and coronary sinus blood samples were then taken every 15 seconds for 2 minutes and every 30 seconds for the next 2 minutes after the intravenous injection in a bolus of 1.0 mCi thallium-201 in saline into the left external jugular vein. After the blood was collected, the animal was sacrificed, the heart removed, eight 1-2 gram samples of free left ventricle were used for counting tissue content of thallium-201. Blood samples were replaced with

equal volumes of normal saline. Blood pressure and EKG were monitored throughout the procedure. All samples were analyzed for sodium and potassium by flame photometry with an Instrumentation Laboratories flame photometer.

The following groups of animals were studied:

1. Control (10 animals)

Each animal was surgically prepared and allowed to stabilize (blood pressure and heart rate) for at least 30 minutes before the thallium-201 was injected.

2. Acute lasix (9 animals)

Each animal was surgically prepared and allowed to stabilize as in the control. 2mg/kg furosemide was then injected in a bolus intravenously via the left jugular vein. At its peak effective time (30 minutes post injection), the uptake of potassium (thallium-201) was investigated by injecting the bolus of 1.0 mCi of thallium-201 and collecting the above described carotid artery and coronary sinus blood samples, and then the ventricular tissue samples.

3. Chronic lasix-normal serum potassium (7 animals)

Each animal was injected intravenously with 1-2 mg/kg furosemide per day for 9 days with an average total dose of 10.54 mg/kg while maintained on a regular diet (Purina Dog Chow and water). Venous blood samples were taken once a week and measured for serum Na and K. Each animal then underwent a control procedure as above.

4. Chronic hypokalemia-no lasix (6 animals)

Each animal was placed on special formula Purina Dog

Chow with only trace potassium. Blood samples were drawn once a week to determine serum potassium. The control procedure was carried out at least one week after the serum potassium was found to be maintained at 3.25 meq/l or less.

5. Chronic lasix-chronic hypokalemia (7 animals)

Each animal was fed the trace potassium Purina Dog Chow and was injected intravenously with 1-2 mg/kg lasix per day for 3-6 weeks until blood samples determined the serum K to be maintained at 3.25 meq/l or less for at least one week. The control procedure was then carried out.

C. DIALYSIS

In addition to being fed trace potassium food, four dogs in group 4 and two dogs in group 5 were dialyzed one to two times to speed the formation of the hypokalemic state. These dogs were dialyzed on a travenol machine against a bath with Diasol without potassium, but with physiologic concentrations of calcium, magnesium, sodium, chloride, phosphate, etc., for 30 to 45 minutes, via jugular venous and carotid arterial lines placed by sterile means and transiently for the dialysis. All dogs were allowed to stabilize for at least two days prior to their use in the preparation, with their serum potassiums measured immediately prior to surgery to ensure the continuance of the hypokalemic state.

D. ANALYSIS OF HEART TISSUE POTASSIUM CONTENT

Two to three samples of 0.5 gram pieces of left ventricle from 13 animals (groups 1,4 and5) were each weighed,

dried for three days in a 100°C oven and reweighed. Each sample was dissolved in concentrated nitric acid for three days, then heated for ten minutes on a heating plate, diluted up to a 10 ml total volume with water, filtered through glass wool and then analyzed for K and Na concentration on an I.L. flame photometer.

VI. RESULTS

A. All Groups

Carotid blood pressure and heart rate remained relatively constant throughout each study once the surgical preparation was completed and the animal allowed to stabilize for at least 30 minutes. Both the arrythmias and subsequent decrease in blood pressure occasionally occuring during the placement of the coronary sinus catheter were transient with a spontaneous return to the previous blood pressure, heart rate and EKG with the coronary sinus catheter securely in place. The hypokalemic dogs (groups 4 and 5) were found to have a higher blood pressure throughout (with means of 225/150 and 190/145, respectively) than the three other groups with a normal serum potassium (with means of 115/89, 120/100, and 170/145, respectively).

B. Left Ventricular Tissue Uptake of Thallium-201

In the control group of 10 dogs, thallium-201 left ventricular myocardial tissue concentration ($\pm$ SEM) averaged 0.0453 $\pm$ 0.0011 % injected dose/gram, (see Table I; Figure 1,2).

In the 9 dogs studied during the peak effect of acute intravenous furosemide (2mg/kg) at 30 minutes post injection, Group 2, myocardial thallium-201 uptake ($\pm$ SEM) was 0.0459 $\pm$ 0.0023 % injected dose which did not significantly differ from control.

Similarly, in the 7 dogs treated chronically with intravenous furosemide over 9 days, in the absence of hypoka-

lemia, Group 3, no significant alteration in thallium-201 myocardial uptake was noted with a mean myocardial tissue uptake of 0.05046 $\pm$ 0.0031 % injected dose/gram.

In the 6 dogs rendered hypokalemic with serum potassium going from 5.13 down to 2.95 meq/l after 3 to 6 weeks on a low potassium diet with or without dialysis, Group 4, mean myocardial thallium-201 concentration ($\pm$ SEM) fell by 8.83% from control to 0.0413 $\pm$ 0.0035 % injected dose/gram. This change in tissue thallium uptake did not significantly differ from the control, acute or chronic furosemide (eukalemic) means. See Figure 1.

However, in the 7 dogs rendered hypokalemic with serum potassium going from 5.21 down to 3.098 meq/l with chronic intravenous furosemide daily and a low potassium diet over a 3 to 6 week period,with or without dialysis, Group 5, myocardial thallium-201 concentration ($\pm$ SEM) fell by 20.31 % to 0.0361 $\pm$ 0.0022 % injected dose/gram ($p < 0.01$ between this group and control, acute furosemide and chronic eukalemic groups.). The results obtained with the hypokalemic dogs were consistent within each group whether or not the dogs were rendered hypokalemic with diet alone or with diet and dialysis.

C. (A-V) Differences (Carotid Artery - Coronary Sinus Blood) (see Table II; figure 3)

Myocardial thallium-201 uptake kinetics as judged by A-V differences paralleled the tissue uptake with no significant differences between the control, acute furosemide and eukalemic chronic furosemide groups. However, the hypokalemic-

no furosemide group, Group 4, had the lowest (A-V) difference and therefore a decrease in myocardial thallium-201 uptake compared to all other groups from sample 4 (one and three-quarter minutes after the thallium injection) to the last sample at four minutes post thallium injection, in particular as compared with control ($p <$ between 0.02 and 0.01), with acute furosemide ($p < 0.05$-0.01), with eukalemic chronic furosemide ($p <$ between 0.05 and 0.01) and with chronic hypokalemic chronic furosemide ($p < 0.05$). In the chronic hypokalemic chronic furosemide group which statistically differed from control, acute and eukalemic chronic furosemide, in myocardial tissue concentration of thallium-201, the (A-V) differences and therefore the thallium uptake was statistically different from the other groups only in scattered samples, (three to four minutes post thallium injection) versus control, (two and one half minutes to four minutes) versus acute furosemide, (one and one half, and three to four minutes) versus eukalemic chronic furosemide, and (between one to two minutes) versus hypokalemic-no furosemide group.

D. Potassium Concentration in Left Ventricular Myocardium

The three groups compared showed no significant change in the potassium content in the myocardium of the hypokalemic versus control animals. The control had a mean of 8.0873 $\pm$0.1739 meq/wet tissue; the hypokalemic-no furosemide animals had a mean of 8.2339 $\pm$0.1324 meq/wet tissue and the chronic furosemide-hypokalemic animals had a mean of 8.1165 $\pm$ 0.1718 meq/wet tissue.

VII. DISCUSSION

It was the aim of this study to see if the administration of the potent diuretic, furosemide, commonly used clinically under both acute and chronic situations, would effect the uptake of an ion commonly used as a tracer in cardiac scanning, thallium-201. Since our results showed no alteration from control in thallium -201 uptake and in(A-V) difference in the acute and chronic normo-kalemic situation, we decided to pursue this investigation using chronic furosemide under a hypokalemic environment, which is a common occurrence in the clinical situation. Since our results showed both a significant decrease in tissue (left ventricle) uptake of thallium-201 and a decreased (A-V) difference in CPM/cc/mCi with this group, we wondered if this decreased uptake was due to the hypokalemia alone. We therefore conducted a control for this situation and tested dogs made hypokalemic via diet alone or diet plus dialysis and no furosemide. These latter results were inconclusive for tissue thallium-201 uptake since they demonstrated a trend toward values lower than the control, acute and chronic furosemide-normokalemic dogs, and higher than the one group that differed significantly from control, the hypokalemic chronic furosemide group, Group 5. Therefore this hypokalemic-no furosemide group , Group 4, approached but did not achieve statistical significance from any other group. More studies, currently under way, will be needed to resolve whether this group with hypokalemia alone, will stand as a group by itself, will merge with the control group, or will merge with the one

significantly different group with decreased thallium-201 uptake, the hypokalemic, chronic furosemide group. If the last possibility comes about, it could be interpreted as meaning that it was the hypokalemic environment and not the furosemide which most likely caused the decrease in myocardial thallium-201 uptake. If it trends toward the control group, it would seem likely that a possible interpretation would be that both a hypokalemic environment along with the chronic administration of furosemide is needed to effect the cationic balance and enzyme systems enough to result in a decreased uptake of thallium-201 by the heart. If, with more studies, it stands as a group by itself, and becomes significantly different from and in between both the control and hypokalemic,chronic furosemide groups, it would lend itself to the interpretation that several factors, each contributing to the whole, influence thallium-201 uptake by the heart, and the more of these factors (hypokalemia, chronic furosemide, etc.) are on board in the environment, the less thallium-201 uptake will occur.

Of considerable interest are the (A-V) difference results of the various, with the hypokalemic only group (Group 4) showing a significantly smaller (A-V) difference, indicating the smallest uptake of thallium-201, than every other group including the hypokalemic, chronic furosemide group (Group 5) although only in scattered samples. This would indicate that these future studies will probably result in the hypokalemic group (Group 4) either standing on its own or merging with the hypokalemic chronic furosemide with the above interpretations applicable.

Although the biochemical mechanisms governing thallium-201 uptake, along with how various environmental factors may alter this uptake, have not as yet been resolved, some interpretation of how studies presented earlier in this paper, may be relevant to the results of this study will be attempted.

It has been shown by numerous investigators that an environment with low external serum potassium (hypokalemia) results in a decreased uptake of Na^+ and K^+ by kidney tubules and red blood cells (32,39,40,41,42). Since thallium-201 has been shown to act as potassium in vivo (1,2,8,9) it would not be unexpected to get a decrease in tissue uptake of thallium-201 in a hypokalemic environment. In addition, studies have shown furosemide to inhibit cation co-transport and block uptake of Na^+ and K^+ in various tissues (red blood cells, renal tubules, salivary duct epithelium)(7,31,32,39,40).

To further support the idea that furosemide directly inhibits potassium uptake by the heart, Seller in 1975 (7) found that furosemide significantly increased the digitalis induced myocardial loss of potassium (as measured by increased coronary sinus-femoral artery potassium difference).

Thus furosemide may act synergistically with the hypokalemia and lead to a decrease in thallium-201 uptake by the heart.

If furosemide inhibits myocardial potassium uptake as the studies seem to show, one may wonder why the acute and chronic administration of furosemide without hypokalemia did not lead to a decreased tissue uptake of thallium-201 or

a decrease in (A-V) difference. One may speculate that a higher serum concentration of furosemide might be needed to see an effect and/or that the normal to high serum potassium of 5.1 in the non-hypokalemic animals may have overridden the effect that might be seen with furosemide alone. The chronic furosemide together with low serum potassium would leave no opposing force to the furosemide's inhibition to potassium, or in this case, thallium-201 uptake.

It is possible that with the cardiac tissue retaining its potassium content, while the serum and skeletal muscles show concomitant reductions in potassium, when made chronically potassium deficient via diet, as shown by Poole-Wilson in 1975 (59), and Cameron in 1975 (61), and this study, that the potassium deficient serum and skeletal muscles may act as a sink for the bolus of thallium-201 injected, thus leading to decreased myocardial uptake of this ion, with none of the projected effects on enzyme systems actually occurring as speculated above.

Since the localization of an infarct with thallium-201 imaging agent is dependent on the lack of perfusion to the infarcted area with a subsequent lack of thallium-201 uptake in that area and thereby the visualization of a "cold spot", the occurrence of a decreased uptake of thallium-201 into normal myocardium under various conditions (hypokalemia and furosemide) would obscure the differences from the abnormal zones under these conditions.

In addition to the finding that acutely reduced serum potassium leads to reduced potassium uptake by kidney tubules and erythrocytes, Erdman in 1971 (62) and Bluschke in 1976(61)

found that 12 days of hypokalemia from diet significantly enhanced the activity of cardiac Na^+,K^+-ATPase which would presumably lead to an increased myocardial potassium uptake whereas this ATPase of the kidney and brain showed no significant change in activity from controls. The same result was found with 14 days of subcutaneous digoxin (0.3 mg/kg), an inhibitor of this ATPase. As the activity of the Na^+,K^+-ATPase is dependent on the potassium concentration and as cardiac glycosides are the specific inhibitors of this enzyme, the observed increase in activity may be explained by an adaptive reponse to the inhibition of the ATPase (61). This increased ATPase activity would presumably return the myocardial potassium uptake toward normal in the face of its inhibitors (hypokalemia and/or digoxin).

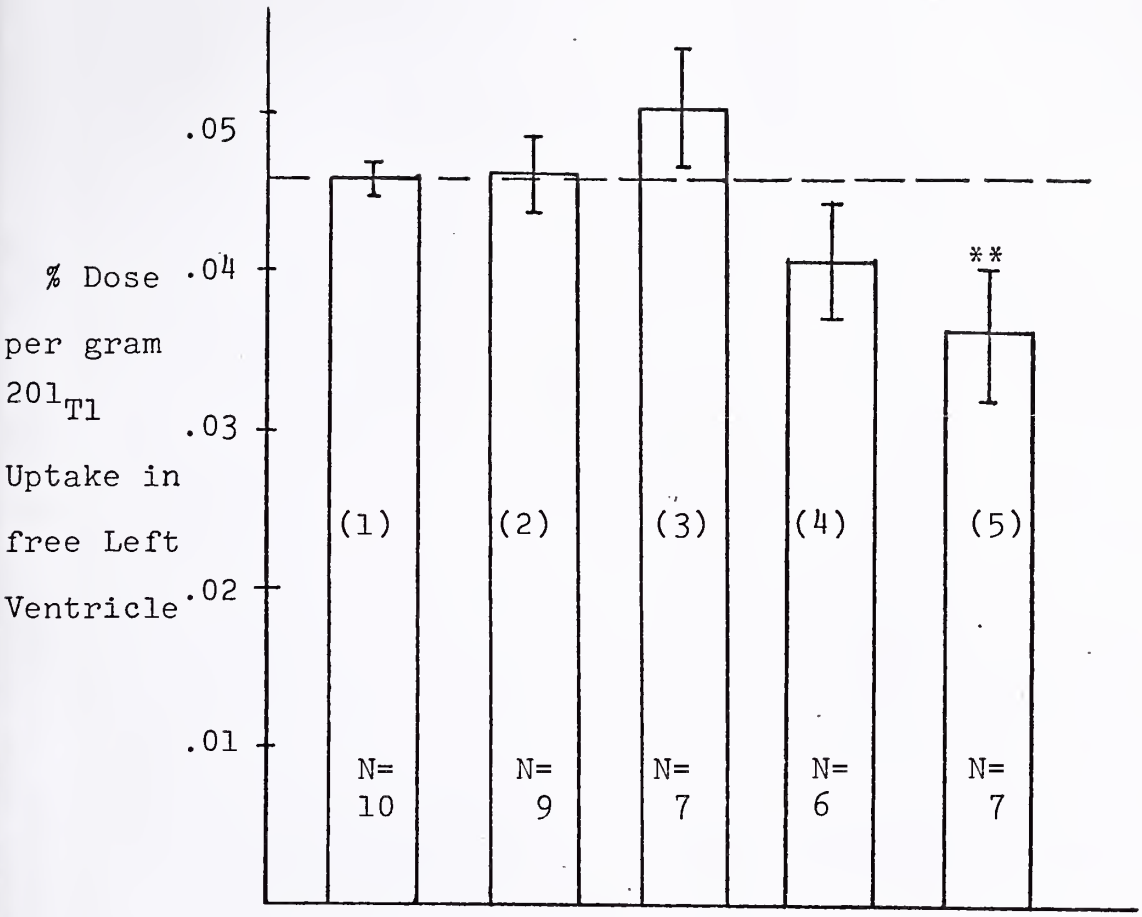
To relate these findings to the present study, it is possible that the hypokalemic alone group, Group 4, had increased cardiac Na^+,K^+-ATPase activity due to the chronic hypokalemia which in turn prevented the expected decrease in thallium-201 uptake (also due to the decrease in external serum potassium). It is possible that this group's intermediate mean value for tissue uptake of thallium-201 was due to these opposing factors. In the chronic hypokalemic chronic furosemide group, Group 5, this possible increase in ATPase activity may have been inhibited by the action of the furosemide leading to the significant reduction in myocardial uptake of thallium-201. If this was so though, one must question why the chronic furosemide alone did not result in an even

greater reduction of uptake. One might also speculate as to whether furosemide (itself an ATPase inhibitor) might also lead to an increase in the activity of this ATPase if given chronically, as did the digoxin.

In the view of this author and Seller (7), it is more probable that the furosemide and hypokalemia may more directly effect the sites where the potassium and thallium-201 act on the myocardium than the above study on the induction of the Na^+,K^+-ATPase.

From this present study, it is possible to make several inferences on how the information gained may be useful in the clinical situation when quantifying thallium-201 scans or looking for relative differences between scans. Let us suppose that a patient is admitted with a diagnosis of acute myocardial infarction or is to have an myocardial infarction ruled out, and it is decided that a thallium-201 scan would be an appropriate tool in his management, it would be most important in the quantitative interpretation of that and subsequent scans to know if he is currently hypokalemic and/or taking medications such as furodemide, on a long term basis or even acutely. From this study, we would expect his scanning results to be the same as control as long as the patient has a normal serum potassium,no matter what his history of taking furosemide. If he has been chronically on furosemide and is hypokalemic alone, then the uptake of the myocardial thallium-201 will be influenced. If normal myocardial tissue is influenced by these various environments to have a decrease in thallium-201 uptake then

the contrast between normal and abnormal regions will be decreased resulting in an inability to detect a lesion or the attenuation of the results.



Decrease in thallium-201 Uptake in Canine Free Left Ventricle under control, acute and chronic furosemide and under normal and hypokalemic conditions. The numbers in the lower part of each column represents the number of experiments of each kind. The number in the parenthesis is the group number.

$^{**}p < 0.01$

(1) = control

(2) = acute furosemide

(3) = chronic furosemide

(4) = hypokalemia alone

(5) = hypokalemia and furosemide

FIGURE 2 % Dose/cc Carotid Artery

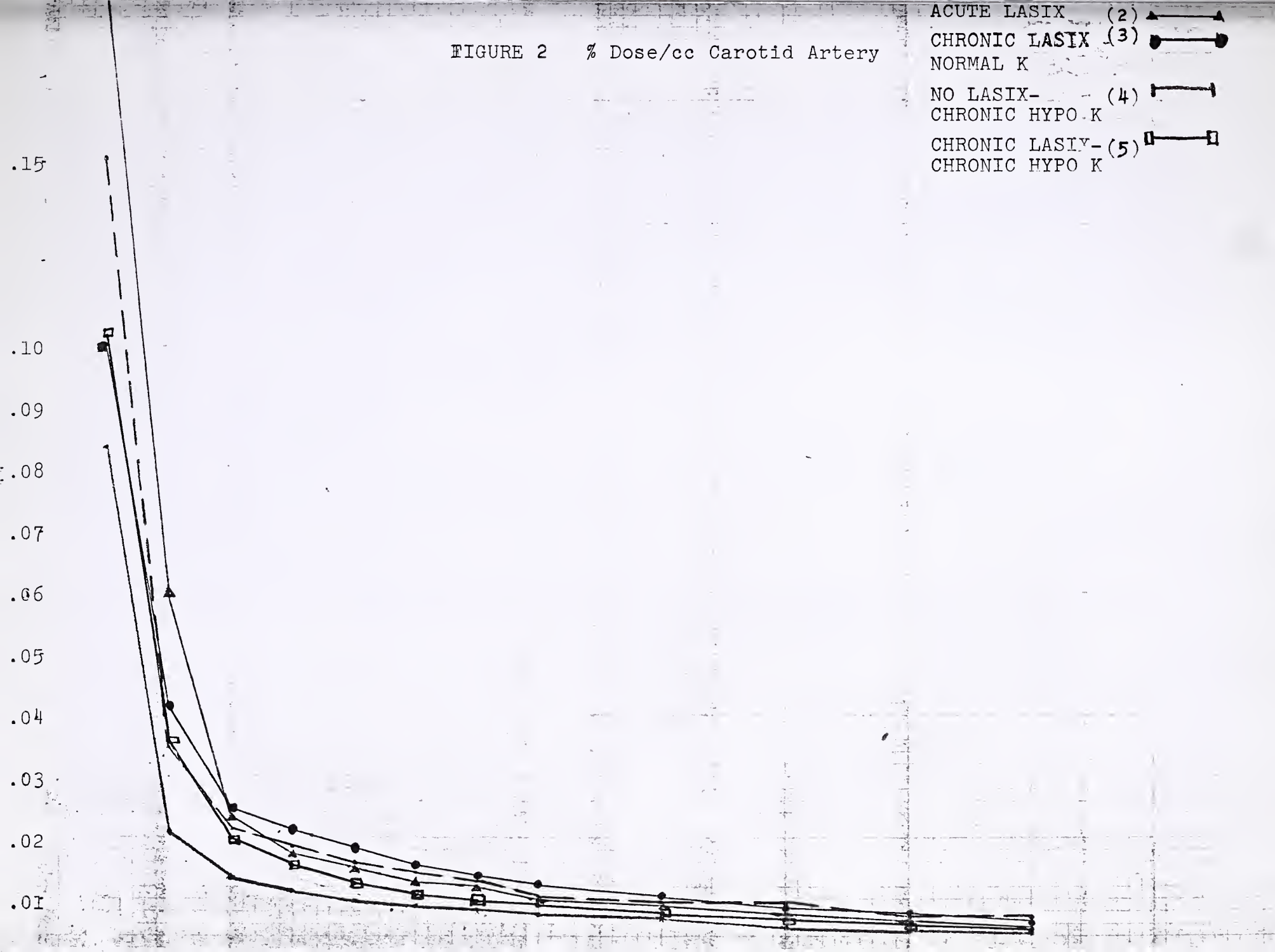


FIGURE 3 (A-V) CPM/cc/mCi/20 kg

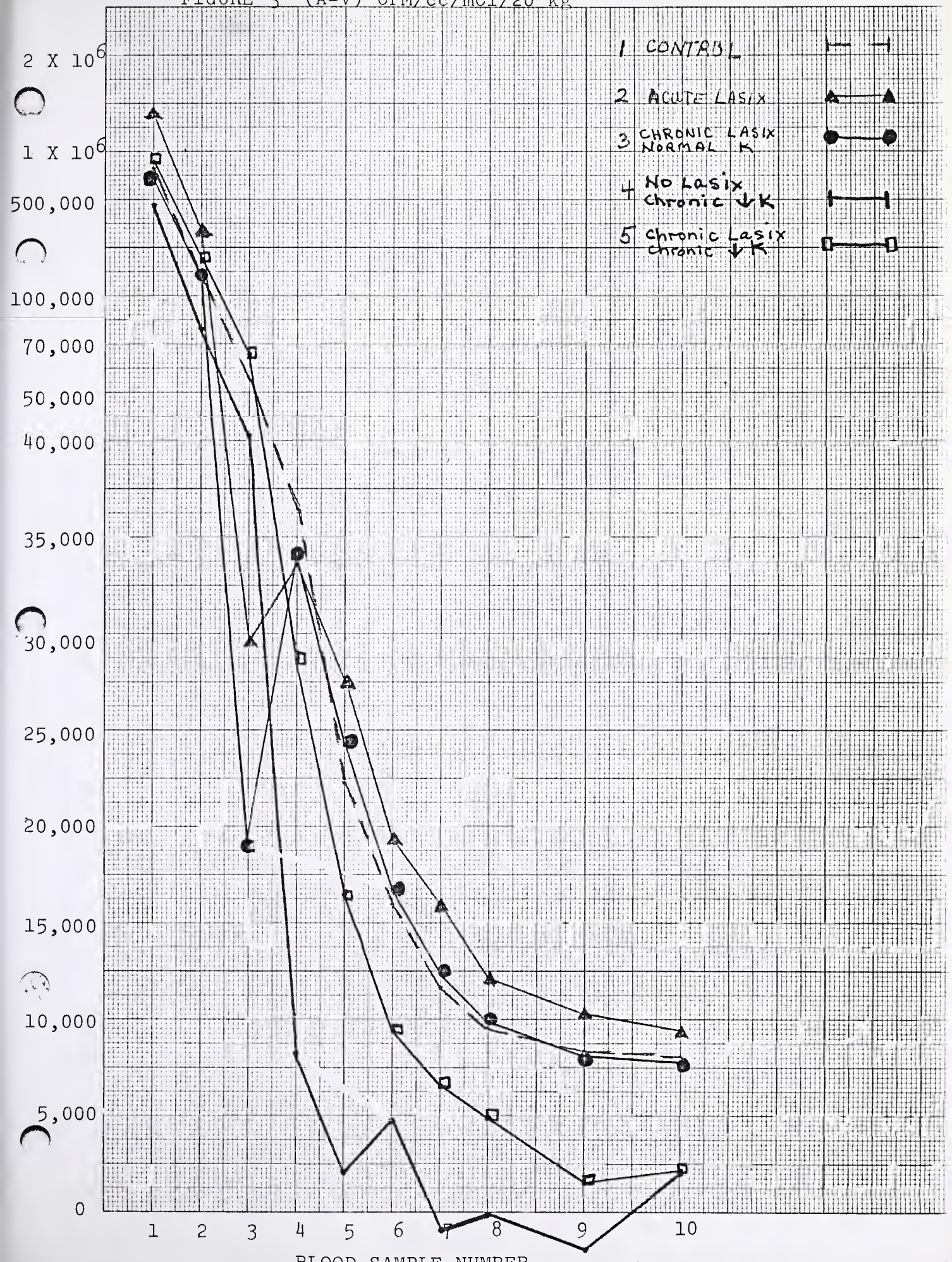


TABLE I % INJECTED DOSE/CC ± SEM OF CAROTID ARTERY

	Blood Sample Number												Heart Tissue
	1	2	3	4	5	6	7	8	9	10	11	12	%Dose/g
CONTROL	0.1516 ±0.0419	0.0355 ±0.0073	0.0217 ±0.0036	0.0184 ±0.0023	0.0160 ±0.0018	0.0142 ±0.0017	0.0129 ±0.0016	0.0112 ±0.0013	0.0098 ±0.0011	0.0093 ±0.0014	0.0074 ±0.0008	0.0074 ±0.0011	0.0453 ±0.0011
ACUTE FUROSEMIDE	0.1968 ±0.0204	0.0612 ±0.0122	0.0236 ±0.0041	0.0177 ±0.0027	0.0151 ±0.0022	0.0128 ±0.0019	0.0119 ±0.0018	0.0103 ±0.0015	0.0090 ±0.0012	0.0077 ±0.0009	0.0068 ±0.0008	0.0058 ±0.0007	0.0459 ±0.0023
CHRONIC FUROSEMIDE-NORMAL SERUM K	0.1038 ±0.0304	0.0418 ±0.0104	0.0250 ±0.0032	0.0214 ±0.0031	0.0182 ±0.0026	0.0158 ±0.0021	0.0138 ±0.0020	0.0121 ±0.0017	0.0104 ±0.0015	0.0088 ±0.0012	0.0077 ±0.0011	0.0067 ±0.0011	0.0505 ±0.0031
CHRONIC HYPOKALEMIA-NO FUROSEMIDE	0.0839 ±0.0219	0.0210 ±0.0037	0.0139 ±0.0021	0.0115 ±0.0013	0.0098 ±0.0011	0.0089 ±0.0010	0.0082 ±0.0010	0.0076 ±0.0010	0.0066 ±0.0008	0.0059 ±0.0006	0.0050 ±0.0005	0.0043 ±0.0005	0.0413 ±0.0035
CHRONIC HYPOKALEMIA-CHRONIC FUROSEMIDE	0.1195 ±0.0178	0.0359 ±0.0064	0.0200 ±0.0021	0.0153 ±0.0011	0.0125 ±0.0011	0.0108 ±0.0008	0.0098 ±0.0007	0.0090 ±0.0006	0.0079 ±0.0004	0.0068 ±0.0004	0.0060 ±0.0003	0.0053 ±0.0002	0.0361 ±0.0022

TABLE II (A-V) = (CAROTID ARTERY - CORONARY SINUS) (± SEM)

	Blood Sample Number									
	1	2	3	4	5	6	7	8	9	10
CONTROL	842,618 ±235,670	129,876 ± 34,005	55,847 ±13,900	36,497 ±16,740	22,319 ± 3,642	15,911 ± 3,402	11,703 ± 2,543	9,531 ± 1,805	8,338 ± 2,067	8,160 ± 2,187
ACUTE FUROSEMIDE	1,460,810 ±186,862	297,758 ± 58,835	29,669 ±21,700	33,606 ± 5,580	27,510 ± 4,484	19,379 ± 4,175	16,079 ± 3,424	12,401 ± 2,594	10,366 ± 1,947	9,478 ± 1,840
CHRONIC FUROSEMIDE-NORMAL SERUM K	707,111 ±234,490	148,004 ± 58,835	19,023 ±24,976	34,106 ± 7,913	24,250 ± 4,044	16,754 ± 2,517	12,439 ± 3,309	9,709 ± 2,413	8,200 ± 2,563	7,756 ± 2,140
CHRONIC HYPOKALEMIA-NO FUROSEMIDE	487,774 ±171,743	81,551 ± 38,611	41,088 ±28,524	8,215 ± 6,666	1,809 ± 3,214	4,720 ± 5,061	-1,415 ± 2,469	-129 ± 1,119	-2,204 ± 2,804	2,225 ± 2,446
CHRONIC HYPOKALEMIA-CHRONIC FUROSEMIDE	890,336 ±148,591	175,442 ± 56,877	66,058 ±12,357	28,781 ± 4,974	16,632 ± 3,597	9,485 ± 1,462	6,748 ± 1,380	4,995 ± 1,438	1,676 ± 742	2,195 ± 1,016

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